AN UPDATE ON FIP

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KEY LEARNINGS/OUTCOMES

After reading this article, you should:

- Understand the relationship between feline coronavirus and feline infectious peritonitis (FIP), and how this impacts transmission and diagnosis
- Be able to list commonly identified findings on history, physical examination and routine clinicopathological tests that raise concern for FIP in a sick cat
- Know how to diagnose probable FIP in a suspected case, and how to definitively diagnose FIP when this is necessary
- Be aware of the current treatment options for a cat diagnosed with FIP, including recent advancements in this field
- Be able to discuss possible methods to reduce risk of FIP within an multicat household

MANUSCRIPT

Background

Feline coronavirus (FCoV) infection in cats is common, usually only causing mild intestinal signs such as diarrhoea. It is highly infectious and found worldwide. A sequela of FCoV infection, feline infectious peritonitis (FIP) is a common cause of death in young cats, occurring in up to 10% of cats infected with FCoV. Although suspicion of FIP is frequent in sick, particularly young, cats, obtaining a definitive diagnosis using non- or minimally-invasive approaches is difficult.

Epidemiology

Coronaviruses are relatively large, enveloped, positive-sense, single-stranded RNA viruses (see **Figure 1** and **Figure 2**). They exhibit a high rate of mutation during replication and therefore exist as clusters of genetically diverse populations. Cats worldwide have been found to be infected with FCoV, with the exception of cats on a small number of isolated islands.

Two serotypes of FCoV are recognised: Type 1, which represents the vast majority of field strains, and Type 2. The latter arises following recombination events between Type 1 FCoV and canine coronavirus. The two serotypes are distinguished primarily by differences in their transmembrane spike (S) glycoprotein. The S glycoprotein (see **Figure 2**) mediates binding to and entry of host cells.

Infection with FCoV is very common, with 35% of the owned domestic cat population having detectable antibodies to FCoV indicating exposure (combined data from the eight

serological studies listed in (Drechsler et al., 2011)). In single cat households (combined data), seroprevalence reduces to 21%, but correspondingly in multi-cat households it can be over 90% (Addie et al., 2000). Most infections are transient (although reinfection is common) with only a small percentage becoming persistent 'carriers' or 'chronically shedding' cats (Kipar and Meli, 2014).

Transmission and Pathogenesis

Transmission is primarily faeco-oral, with litter boxes representing the principal source of infection amongst cats within a household. In breeding catteries, kittens commonly become infected at a young age, mostly at 5-6 weeks (Addie and Jarrett, 1992), as maternally derived antibodies have started to wane. Nose to nose contact is considered an uncommon route, and transplacental is considered rare. Experimentally, infection has been transmitted by parenteral injection of virus derived from cats with FIP.

Small intestinal villi enterocytes are the primary point of host cell entry and replication. In most cases, FCoV infection is subclinical or results in only mild gastrointestinal signs (e.g. diarrhoea, vomiting). However, occasionally more severe gastrointestinal disease is seen. Subclinical FCoV infection was previously believed to be confined to the intestinal tract, but we now know that healthy FCoV-infected cats develop a detectable low-level viraemia during acute infection (Kipar et al., 2010). In a small percentage of cases FCoV infection results in feline infectious peritonitis (FIP), which typically occurs sporadically. Occasional outbreaks of FIP in multi-cat households or shelters affect a larger percentage of cats (Barker et al., 2013).

In FIP, virus-laden monocytes attach to the walls of small veins and release inflammatory cytokines that damage the endothelial basal lamina (Kipar and Meli, 2014). This results in extravasation of monocytes (which mature into tissue macrophages) and proteinaceous fluid. In effusive (a.k.a. 'wet') FIP, this extravasation of proteinaceous fluid is evident as fluid accumulations within body cavities. In non-effusive (a.k.a. 'dry') FIP, the extravasated macrophages recruit other inflammatory cells and result in perivascular granulomata, which may appear grossly as a mass lesion (**Figure 3** and **Figure 4 A**). The role of other cytokines, including interferon gamma and tumour necrosis factor-alpha (TNF-α), in the pathogenesis of FIP is incompletely understood, but is thought to be significant (Kipar and Meli, 2014). The mesenteric lymph nodes (MLNs) may represent an important site in which the host immune response to FCoV plays a role in the outcome of infection, as MLNs are presumed to be the first site of FCoV replication outside the intestinal tract and before monocyte/macrophage infection occurs (Malbon et al., 2019).

Viral factors are important in the pathogenesis of FIP. As mentioned earlier, the S glycoprotein of FCoV mediates host cell entry, with mutations in the S gene influencing cell tropism (Kipar and Meli, 2014). Mutations at different sites within the S gene have been detected with increased frequency in FIP tissue-derived FCoVs, as compared to faecally-shed FCoV from clinically 'healthy' cats (Chang et al., 2012, Licitra et al., 2013). This has led to suggestions that some of these mutations could be a useful target in differentiating cats with FIP from cats without. Unfortunately, a recent large-scale study suggested that one of these sets of mutations, involving the fusion peptide, was more indicative of systemic FCoV infection, occurring in FCoV viraemic cats with and without FIP with equal frequency, rather than FIP per se (Barker et al., 2017). Other viral factors mediating effective and sustained

replication in monocytes, and activation of infected monocytes, are also likely to be important for the development of FIP following systemic FCoV infection. Very recently there has also been suggestion that specific viral mutations could be associated with tissue tropism (Andre et al., 2019).

Host factors contributing to the immune response such as genetic background (e.g. breed-, line- or individual-specific) and maturity (e.g. age; history of prior exposure to infectious agents) likely play an important role in FIP development. Host factors are inextricably linked with environmental factors such as stress (e.g. cat-cat interactions; novel experiences such as rehoming, vaccination, surgery; resource accessibility) and overcrowding, which themselves may lead to increased environmental viral burden, increased viral replication within cats and support FIP development.

Clinical signs associated with FIP

The variability in the extent and distribution of both vasculitis and perivascular granulomata underlies one of the difficulties in diagnosing FIP. Clinical signs of FIP can change over time, necessitating repeated clinical examinations to detect newly apparent pathology. Non-specific, often waxing and waning, clinical signs (see **Table 1**) attributable to the systemic inflammatory response frequently occur in cats both with and without detectable effusions, with FIP a significant differential for pyrexia of unknown origin (Spencer et al., 2017). Although, it should be noted that the absence of these signs does not rule out FIP.

Table 1 Commonly encountered features of signalment, history, and physical examination seen in cats with feline infectious peritonitis.

Signalment	**Young** (often <2 years); male; breed*
History	*Background*: **Recent stress** (vaccination; rehoming; new cat; surgery); multicat household (current / historical) *Health*: **weight loss / failure to thrive**; **inappetence / anorexia**; **lethargy**; **pyrexia of unknown origin** (non-responsive to antibiotics; +/- fluctuating); behavioural change, ataxia, seizures
Physical examination	Abdominal distention / fluid thrill [***ascites***]; **palpable mass**; **uveitis**; **jaundice**; **pyrexia**; restrictive dyspnoea with dull lung sounds [***pleural effusion***]; neurological deficits; lymphadenopathy

* Non-pedigree cats make up the majority of cats presenting with FIP (80% in a recent study (Richards, 1995)). However, various prevalence studies have identified increased incidence in certain pedigree breeds. The breeds identified of having increased risk vary from country to country, suggestive of either country-specific blood lines being more of a factor or reporting bias within the local pedigree cat communities.

Although effusive FIP is regarded as being 3-4 times more common than non-effusive FIP (Kipar and Meli, 2014, Riemer et al., 2016), and the distinction between the two forms is important for diagnostic purposes, there is considerable overlap between them. Cases with effusive FIP often have pyogranulomatous lesions visible at post-mortem examination, whilst many cats with non-effusive FIP go on to develop effusions. Effusive FIP is often acute in nature, progressing within a few days or weeks; whereas non-effusive FIP tends to be more chronic, progressing over a few weeks to months. In effusive FIP, effusions may form in one or more body cavity, with abdominal effusion leading to a clinical presentation of ascites and abdominal distension being the most common manifestation. Cats with pleural

effusion often develop dyspnoea, whereas cats with pericardial effusion rarely show signs of cardiac tamponade. Occurring only rarely, scrotal effusion leads to scrotal enlargement in entire males. Non-effusive FIP is often more difficult to diagnose, particularly in the earlier stages of disease, as vague non-specific signs may be all that can be seen. More specific signs depend on the organs affected by the granulomatous lesions, often the central nervous system (CNS), eyes, or abdominal organs (e.g. liver, MLNs, kidney, gastrointestinal tract); however, any tissue can be affected and primary involvement of the lungs or skin have been described.

In sick cats, careful neurological and ocular examination may reveal changes that support a diagnosis of FIP, as well as indicating a potential source of samples for testing. Neurological signs associated with focal, multifocal or diffuse changes in the CNS may be seen in up to 30% of cats with FIP, and for some these are the only signs noted (**Figure 5**); this makes FIP a common differential for neurological disease, particularly in the young cat. Commonly reported signs in FIP with neurological involvement include ataxia (with varying degrees of tetra- or paraparesis), hyperaesthesia, head tilt, nystagmus, seizures, behavioural change, mental state change, cranial nerve deficits and postural reaction deficits; however, differentiating subtle neurological signs from those exhibited by systemically unwell cats may not be possible. Similarly, FIP is a major differential for uveitis (**Figure 6**) with anterior and posterior uveitis commonly identified in cats with both effusive and non-effusive FIP, particularly when examined by an experienced clinician.

Where FIP manifests in the intestinal tract and/or regional lymph nodes (sometimes called "focal FIP", although the disease is still systemic) it can present as a palpable abdominal

mass (see **Figure 4**) that must be differentiated from neoplasia, toxoplasmosis or other granulomatous disease (e.g. mycobacterial infection) (Kipar et al., 1999, Pedersen, 2009). Where the lesion involves the intestinal wall clinical signs may include vomiting, diarrhoea or constipation, or signs referable to an obstructive or protein-losing enteropathy may be seen.

Diagnosis (see **Figure 7**)

A high index of suspicion can be obtained from a combination of signalment, history, and physical examination (**Table 1**). However, none are pathognomonic for FIP and other common differential diagnoses (**Table 2**) should be considered when performing further investigation.

Routine clinicopathological tests (**Table 3**) may indicate the presence of a chronic systemic inflammatory response, further supporting a clinical suspicion of FIP; however, no clinicopathological changes are diagnostic for FIP and, in some cats, routine blood analysis can be unremarkable. This is compounded by both vets (and owners) suspecting FIP earlier in the course of the disease process, so reducing the negative predictive power of some findings (e.g. absence of hyperglobulinaemia) (Stranieri et al., 2017).

177 **Table 2** Common differential diagnoses for feline infectious peritonitis

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	Non-specific signs	Jaundice	Effusion	Ocular	CNS	Mass lesion	Notes
Toxoplasmosis	✓	✓	✓	✓	✓	✓	*History* – Fed raw diet or hunter (also vertical transmission in kittens) *Differences* – Hyperglobulinaemia uncommon *Diagnosis* – Cytological identification of organisms on aspirates; PCR of aspirates or CSF; paired *Toxoplasma* serology (IgM & IgG)
Lymphocytic cholangitis	✓	✓	✓ (ascites)				*History* – Persians may be over-represented *Differences* – Usually (not always) associated with increased hepatic enzyme activities (primarily cholestatic). Cats often relatively well and normothermic *Diagnosis* – Liver biopsy
Neoplasia (e.g. lymphoma; carcinoma)	✓	✓	✓	✓	✓	✓	Can affect cats of any age, particularly lymphoma. Jaundice may be present particularly with hepatic involvement *Diagnosis* – Cytology of fluid or aspirates; biopsy
Mycobacterial disease	✓		✓	✓	✓	✓ (often LNs)	*History* –Hunter or outdoor access (geographical variation), fed raw diet *Differences* – Usually minimal to no effusions. Usually (not always) relatively well and normothermic. Pulmonary signs (tachypnoea; cough) not uncommon *Diagnosis* – Ziehl-Neelsen stain of aspirates or biopsy; interferon-γ release assay; mycobacterial PCR or culture of aspirate or biopsy

Pancreatitis	✓	✓	✓ (ascites)			✓ (pancreas)	*Differences* – Usually (not always) normothermic. Ascites, where present, usually small volume with high cellularity (non-degenerate neutrophils) *Diagnosis* – Feline pancreatic lipase immunoreactivity; abdominal imaging
Feline immunodeficiency virus (FIV) / feline leukaemia virus (FeLV)	✓			✓	✓	✓ (LNs)	*History* – Outdoor or 'stray'; entire adult with unknown mating activity (especially FIV) *Differences* – Common differential for lymphadenopathy and/or uveitis. FeLV may be associated with neoplasia (especially lymphoma) *Diagnosis* – FIV antibody / FeLV antigen serology (positive results should be confirmed)
Sepsis	✓	✓	Infection can involve different organ systems (e.g. kidney; liver; uterus; heart) or body cavities (e.g. pyothorax; septic peritonitis)				Cats are often very sick, e.g. pyrexia may have progressed to hypothermia with onset of shock *Diagnosis* – haematology suggestive (leukocytosis or neutropenia; left shift and toxic change); hypoglycaemia may be present; imaging; cytology (degenerate neutrophils; intracellular bacteria) and culture of fluid or aspirates*
Septic peritonitis	✓		✓ (ascites)				Pyrexia common. Most frequently associated with gastrointestinal or urinary tract perforation *Differences* – Ascites with high cellularity (degenerate neutrophils; intracellular bacteria) *Diagnosis* – cytology and culture of fluid or aspirates*
Pyothorax	✓	✓	✓ (pleural)				Usually pyrexic *Differences* – Pleural effusion with high cellularity (degenerate neutrophils; intracellular bacteria)

							Diagnosis – cytology and culture of fluid or aspirates*
Congestive heart failure (CHF)	✓		✓ (pleural +/- ascites)				*History* – *S*ome breeds are predisposed to cardiomyopathy (e.g. Ragdoll; Maine Coon) with increased risk of CHF at a young age. Heart murmur (non-haemic), gallop sounds, arrhythmia, jugular vein distention and pulse may be present. *Differences* – Low protein / low cellularity effusion. Hypothermia and/or hypotension are common. Pyrexia, hyperglobulinaemia and jaundice are not features *Diagnosis* – Echocardiography

179 ✓ = feature shared with FIP (NB: absence does not rule it out as a differential); * NB: risk of false-negative if collected after antibiotics

180 administered; LNs = lymph nodes

Table 3 Commonly encountered changes on routine clinicopathological analysis seen in cats with feline infectious peritonitis.

Haematology	**Anaemia** (often mild & non-regenerative) **Microcytosis** **Lymphopenia** **Neutrophilia** (+/- left shift)
Serum biochemistry	**Hyperglobulinaemia*** (often polyclonal gammopathy) Hypoalbuminaemia (secondary to acute phase protein response; compensatory for hyperglobulinaemia **Hyperbilirubinaemia** **Low albumin to globulin ratio** Increased liver enzyme activities (primarily hepatocellular, esp. AST)

Further support for FIP may be gained from the measurement of inflammatory markers. Serum protein electrophoresis is a crude way of determining the presence and nature of an inflammatory response, particularly where there is a hyperglobulinaemia. The most frequently encountered change in cats with FIP is a polyclonal gammopathy, indicating a non-clonal increase in antibodies; however, a small number of cats present with a monoclonal gammopathy (Taylor et al., 2010), whilst others show increases in the alpha2-globulin fraction (reflecting an increase in acute-phase proteins (APPs))(Stranieri et al., 2017). APPs are made in the liver in response to cytokines released from activated macrophages and monocytes. Marked increases (>1.5 mg/mL) in serum α1-acid glycoprotein (AGP) can support a diagnosis of FIP (Paltrinieri et al., 2007, Duthie et al., 1997, Hazuchova et al., 2017). Other APPs, serum amyloid A and haptoglobin, have been assessed in the diagnosis of FIP but were both less sensitive and specific than AGP (Duthie et al., 1997, Hazuchova et al., 2017). Overall, increased AGP (or other APPs) in serum, despite

197 supporting a diagnosis of FIP, is not confirmatory and may be limited by cost, availability and
198 turnaround time.
199
200 Clinicians vary as to whether they perform FCoV serology or not in suspected cases.
201 Although a positive result indicates exposure to FCoV, many clinically healthy cats have
202 positive, often high, antibody titres, whilst a small proportion of cats with both effusive and
203 non-effusive FIP are seronegative. Diagnosis of FIP should never be made based upon
204 positive serology alone. Faecal RT-qPCR has replaced serology in monitoring the effect of
205 control measures in the management of FCoV infection within a breeding cattery.
206
207 Imaging (see **Figure 4** and **Figure 5**) can be useful, in that it can often identify areas of
208 pathology (e.g. mass lesion; effusion) that may prove useful to sample as well as guiding
209 sample acquisition (e.g. ultrasound-guided needle biopsy). However, imaging alone cannot
210 be used to make a diagnosis of FIP.
211 To provide a definitive diagnosis of FIP, cytological or histopathological changes consistent
212 with FIP (i.e. pyogranulomatous inflammation) should be identified and subsequently co-
213 localised with FCoV antigen, using immunostaining for viral antigen. More recently RT-PCRs
214 have also been used to support a diagnosis of FIP (see **Box 2**).
215
216 In effusive FIP, sampling the effusion is the single most useful diagnostic step in confirming a
217 diagnosis. For this reason, where effusions are not evident on initial evaluation, repeated
218 ultrasonography to identify any small volume effusion is recommended (**Figure 4**) and may
219 facilitate sampling of small pockets of fluid. FIP effusions (**Figure 8**) are *usually* clear, poorly
220 cellular (total nucleated cell count $<5x10^9$/L), yellow, viscous, protein-rich (with a total

protein concentration of >35g/L), have a low albumin to globulin ratio, and have a positive Rivalta test (**Box 1**). However, in some cats the effusions might be cloudy, of slightly lower protein levels (e.g. in cats that were not originally markedly hyperproteinaemic, or following repeated abdominocentesis), or contain much higher cell counts (up to $20x10^9$/L). Cytological examination usually reveals pyogranulomatous inflammation with macrophages, non-degenerate neutrophils and few lymphocytes. Effusion AGP concentrations may also be useful in supporting a diagnosis of FIP, potentially affording greater sensitivity and specificity than serum measurements (Duthie et al., 1997, Hazuchova et al., 2017), but are not confirmatory. Positive FCoV antigen immunostaining is strongly supportive of a diagnosis of FIP. However, false-negatives occur in 5-43% of cats with FIP (Hartmann et al., 2003, Paltrinieri et al., 1999), particularly in low cellularity samples, and false-positives have been reported in up to 30% of cases (Hartmann et al., 2003, Felten et al., 2017b, Litster et al., 2013), including cats with neoplasia or cardiac disease. False-positive results may be dependent on technique, methodology or laboratory used, therefore checking the specificity and use of internal controls with the laboratory used is recommended when interpreting results.

Box 1: *The Rivalta test*

This test is a simple inexpensive, point-of-care test to differentiate a transudate from an inflammatory effusion. It does not replace more advanced analysis in-house or at external laboratories, but it can facilitate decision-making particularly in financially constrained settings, particularly where tests to quantify effusion protein concentrations are not available.

Rivalta test is more useful at ruling out FIP, than ruling it in: over 90% of FIP effusions are Rivalta test positive (Hartmann et al., 2003, Fischer et al., 2012); positive results are also seen with bacterial peritonitis and neoplastic exudates; and low protein, non-inflammatory exudates (such as those see with cardiac failure or hypoproteinaemia) are typically negative.

Method: 10 ml distilled water mixed with 2-3 drops of white vinegar in a test tube or universal container; one drop of effusion is added carefully to the top

* Negative = dispersion of the drop of effusion

* Positive = the drop of effusion retains its shape and floats slowly to the bottom of the tube or sits on the surface of the water

For cats with suspected non-effusive FIP and accessible mass lesions (e.g. mesenteric lymphadenomegaly) fine needle cytology may be considered. Whilst in cats where CNS (see **Figure 5**) or ocular signs (see **Figure 6**) predominate, more specialist techniques to obtain samples of cerebrospinal fluid or aqueous humour for analysis are discussed in the literature but rarely performed in first-opinion practice. Cytology typically reveals non-septic pyogranulomatous to granulomatous inflammation; however, this is only documented in 42 to 82% of cats with FIP, and up to 30% samples from cats without FIP (Gruendl et al., 2017, Felten et al., 2018, Giordano et al., 2005). Positive immunostaining for FCoV antigen can provide further support for FIP. However, as with cytological analysis alone, false-negatives occur >15% of CSF samples (Gruendl et al., 2017), >35% of aqueous humour samples (Felten et al., 2018), and 11-53% of tissue aspirates (Felten et al., 2019, Giordano et al., 2005) from cats with FIP, with false-positives reported in ~20% of samples from cats without FIP, including those with neoplasia or vascular disease.

Although, until recently the reference standard for the diagnosis FIP, histopathology alone can be non-diagnostic, equivocal or misleading in some cases (Pedersen, 2009, Giuliano et al., 2018, Giordano et al., 2005), particularly where needle-core samples are collected blind. Many now consider the demonstration of FCoV antigen within granuloma-associated macrophages by immunostaining as the reference standard, but it is subject to the similar limitations to histology albeit with 100% specificity. In a recent large study, only 62% of tissue samples from cats with FIP revealed FCoV-positive lesions (Barker et al., 2017). However, it should be noted that all the samples in this study were collected post-mortem, visibly normal tissues were frequently sampled in addition to grossly abnormal tissues, and at least one tissue sample per cat was diagnostic for FIP. Wherever possible grossly abnormal tissue should be sampled to maximise the likelihood of achieving a diagnosis.

Box 2: *The use and abuse of reverse-transcriptase PCR (RT-PCR) in the diagnosis of FIP*

FCoV RT-PCR, when designed appropriately, is a very sensitive and specific assay for the detection of FCoV within samples, and is generally more sensitive than immunostaining for FCoV antigen in tissues (Barker et al., 2017). However, it cannot co-localise virus to cytological / histological lesions, merely to the samples in which those changes are present. Following intestinal infection with FCoV, most cats develop a viraemia, disseminating virus throughout the body. Cat without FIP can therefore have detectable virus within blood, effusions and tissues – albeit at a lower frequency and viral copy number. Due to low circulating levels of viraemia, use of RT-PCR of whole blood in cats with suspected FIP is not recommended (Emmler et al., 2019).

In a recent large study, all but one cat of 57 (98%) with FIP had at least one tissue positive for FCoV by quantitative RT-PCR, as compared to 12 of 45 cats without FIP (Barker et al., 2017); viral copy numbers were also significantly higher in the positive samples from cats with FIP than those without FIP. Further investigation of the single cat with FIP and a negative RT-PCR result revealed the FCoV present to have multiple mutations in the sequence normally detected by the RT-PCR assay, resulting in its failure. Whilst, none of the cats without FIP (including those with a positive RT-PCR result) had histopathological evidence of granulomatous disease or positive immunostaining.

RT-PCR has been applied to cytological samples. Most (72-100%) effusions from cats with FIP are RT-PCR positive, cf. only two false-positives out of 76 samples from cats without FIP across three studies (Barker et al., 2017, Felten et al., 2017c, Stranieri et al., 2018). Most (18 of 20; 90%) mesenteric lymph node aspirates from cats with non-effusive FIP are RT-PCR positive (Dunbar et al., 2018); however, one false-positive result (out of 20 cats) did occur in a cat seropositive for FCoV. Detection of FCoV in CSF by RT-PCR from cats with FIP is variable, ranging from 21% to 86% (Barker et al., 2017, Doenges et al., 2016, Foley et al., 1998, Emmler et al., 2019), whereas, RT-PCR was negative in all control cats. Detection of FCoV in aqueous humour from cats with FIP was poor (25%), and no control cats were tested (Emmler et al., 2019).

Additional analysis has been applied to RT-PCR-positive samples to determine whether the FCoV present carries genetic mutations that have been said to be associated with FIP. The use of *Spike* gene mutation analysis has been most frequently studied for this purpose, albeit using different techniques, different sample types and with different conclusions.

Where a highly sensitive method (pyrosequencing) was employed to evaluate the *Spike* gene, mutations were detected in FCoV-positive tissue from 15 of 17 (88%) samples from cats without FIP as compared to 202 of 206 (99%) samples from cats with FIP (Barker et al., 2017). Other techniques (e.g. allelic discrimination) that require a relatively high viral copy number in the sample to generate a result (often not present in cats without FIP) and consider a result where sequencing has failed to be negative, will increase the test specificity by a modest amount by reducing, but not eliminating, the number of false-positives; however, the detection of true-positives results in cats with FIP (i.e. the test sensitivity) is more markedly reduced (Emmler et al., 2019, Felten et al., 2017a).

In conclusion, although a positive RT-PCR result on fluid, effusions, aspirates and tissue can provide strong support for a diagnosis of FIP (particularly for CSF), both false positives and false negatives occur such that RT-PCR should not be solely relied upon to make a diagnosis. Further, *Spike* gene analysis is either of little benefit over RT-PCR at removing false-positives (i.e. when pyrosequencing is used), or markedly increases the number of false-negatives (i.e. when allelic discrimination is used) and may inadvertently cast doubt on a diagnosis of FIP in a with FIP potentially delaying treatment.

NB: RT-PCR of faeces is *not a test for FIP*, it is a test for FCoV shedding (which can be intermittent). Most cats that have a positive faecal FCoV result will not go on to develop FIP, and only two in every three cats with FIP are shedding FCoV at time of euthanasia (Barker et al., 2017). It is only of use in special circumstances (e.g. attempting to identify shedders within a multi-cat household)

Often, and especially in non-effusive FIP, collection of biopsies from tissues with gross lesions is necessary to achieve a definitive diagnosis. In the absence of a definitive diagnosis, or pending confirmatory tests, available results form the basis of discussion as to whether further, invasive, investigation is likely to change treatment options and whether to start treatment. This can been frustrated by the geographical restriction (outside the UK) of some tests (e.g. AGP, immunocytochemistry, immunohistochemistry, and RT-PCR) that would otherwise be strongly supportive of a diagnosis of FIP. If euthanasia is performed without a definitive diagnosis, post-mortem examination is strongly recommended to assess whether gross findings (with histopathology if funds allow) are consistent with a diagnosis of FIP.

Treatment & Prognosis

Potential alternative diagnoses, such as toxoplasmosis and mycobacterial infection, should be ruled out and a definitive diagnosis of FIP made prior to considering treatment; however, the reality is that treatment is often started when as close to a definitive diagnosis of FIP as possible has been achieved, taking into account the overall clinical picture alongside owner preferences and finances. A lack of definitive diagnosis makes it impossible to know whether a treatment response indicates efficacy against FIP, or a missed alternative diagnosis. Treatments administered may also interfere with the sensitivity and specificity of future diagnostic test results. A paucity of placebo- or 'current best-treatment'-controlled clinical trials of cats with definitively confirmed FIP limits treatment recommendations. Currently, no licensed drug is available that has proved effective in curing FIP.

Prognosis for cats with effusive disease is grave, with death or euthanasia within days to occasionally weeks in most cases. The prognosis for cats with non-effusive disease is also

poor, with death or euthanasia within weeks to months in most cases. However, it is not necessary to euthanase immediately if the cat still has a reasonable quality of life. It is possible to maintain palliative treatment for as long as weight and activity are maintained. Rarely some individuals have survived for months to sometimes years, often with supportive treatment, but it is unclear as to whether the treatment administered influenced survival.

Treatment is currently limited to supportive care. Cats, once anorexic, can quickly become dehydrated; therefore, simple fluid therapy, correction of electrolyte disturbances, and encouraging them to eat can be extremely useful at improving their quality of life. The value of removing fluid effusions in cats with FIP has been debated. Thoracocentesis is indicated where effusion has resulted in dyspnoea. Abdominocentesis is controversial and may be detrimental due to exacerbation of dehydration, although some authors have described fluid drainage followed by intracavitary corticosteroid administration.

Given that FIP has a significant immune-mediated component, treatment to either suppress or modify the immune response can be considered. Corticosteroids are the most frequently used medication – and some cats receive benefit from them, particularly in terms of quality of life. However, there are no controlled studies to prove beneficial effect, only anecdotal reports, and they appear to do very little for the viral infection itself. Doses are empirical (prednisolone 2-4mg/kg/day orally) and can be tapered slowly to response.

Many different drugs have been considered for the treatment of FIP. Cyclophosphamide, ciclosporin A and anti-TNF-α antibodies have been anecdotally used to prolong survival, but no controlled studies have been performed. Pentoxifylline has also been anecdotally used to

manage the vasculitis; however, in a placebo-controlled trial of the related drug, propentofylline, no benefit was found (Fischer et al., 2011). Interferons are also commonly used, on the basis of positive anecdotal reports; however, a placebo-controlled clinical trial failed to demonstrate a clinically relevant benefit (Ritz et al., 2007). Polyprenyl immunostimulant has limited data to support its use with significant limitations (including lack of control treatment group and limited diagnostic criteria for FIP) (Legendre et al., 2017); however, it is possible that it *may* improve survival times in the milder forms of non-effusive FIP without detrimental impact on the patient. Herbal medication has also been suggested for cats with FIP, often with no scientific data to support its use.

Recently described promising new, but as yet unlicensed, drugs comprise viral protease inhibitors and nucleoside analogs. FCoVs produce large viral proteins (e.g. the gene encoding polyprotein 1 forms a large component of the FCoV genome, see **Figure 1**) that are cleaved into smaller functioning units by proteases. Inhibitors of these proteases therefore affect viral production. The protease inhibitor GC376 has produced remarkable responses in both experimentally-induced and naturally-occurring FIP, with six of eight cats with experimental-induced FIP alive at 8 months and 19/20 cats with naturally-occurring FIP showing a positive response, which was sustained in seven cats (Kim et al., 2015, Pedersen et al., 2018). The nucleoside analog GS-441524 acts as an alternative substrate and RNA-chain terminator of the viral RNA polymerase, thus interfering with FCoV replication. It too has produced remarkable responses in both experimentally-induced and naturally-occurring FIP, with all 10 cats with experimental-induced FIP alive at 8 months and 26/31 cats with naturally-occurring FIP having a positive response, which was sustained in 25 cats (Murphy et al., 2018, Pedersen et al., 2019). Unfortunately, both the protease inhibitor GC376 and

411 the nucleoside analog GS-441524 appear to poorly penetrate the blood-brain and blood-eye
412 barriers, likely accounting for increased likelihood of relapses involving the nervous system
413 or lack of initial response to treatment in study cats presenting with neurological or ocular
414 signs of FIP. The use of higher than previously reported doses of these agents, along with
415 extended courses, have been suggested for cases of neurological or ocular FIP; however,
416 more studies are warranted.
417
418 The authors are aware that, in the absence of commercially available licensed products,
419 some UK cat owners have obtained black-market forms of both GS-441524 and GC376 via
420 the internet for the treatment of FIP in their pet. By their nature, these black-market
421 products are of unknown quality, efficacy, toxicity and longevity, and therefore cannot be
422 prescribed by veterinary surgeons for their patients.
423
424 A 'nutritional supplement' (Mutian) containing a novel adenosine nucleoside analogue
425 (Mutian® X; reported to be different to GS-441524) has been marketed worldwide, primarily
426 at cat owners, for the treatment of FIP (Addie et al., 2020). However, there is only limited
427 published research describing its use to stop faecal shedding of virus (Addie et al., 2020).
428 There is currently no peer-reviewed evidence base upon which to recommend its use in cats
429 with FIP. Further, according to both the Veterinary Medicines Directorate (UK) and the Food
430 and Drug Administration (USA), nutritional supplements may not be presented with
431 medicinal claims (e.g. the ability to cure cats of FIP), otherwise they would be considered as
432 a veterinary medication requiring authorisation.
433
434 **Prevention and in-contact cats**

One of the most frequent questions from owners following the diagnosis of FIP in one of their cats is what do with the other cats in the household. For the major considerations see **Box 3**. As spread of FCoV is most of a concern amongst large groups, reduction in environmental viral load through improved hygiene is key. Appropriate care in cleaning and use of disinfectants (see (Addie et al., 2015) for more information) to reduce environmental, including fomite, contamination is necessary. This includes maintenance of toileting facilities including: sufficient litter-trays per cat; siting litter-trays away from food and water; and use of cat litter that may have enhanced neutralisation of FCoV to limit fomite spread (e.g. dust-free clumping Fuller's earth litter)(Addie et al., 2019). Although achievement of a FCoV-free household or kittens (e.g. via early weaning) is technically possible, it is not without significant cost and potentially welfare concerns – more information can be found in the latest ABCD guidelines on Feline Infectious Peritonitis (www.abcdcatsvets.org/feline-infectious-peritonitis/).

Minimising host risk factors, particularly relating to stress of conflict or overcrowding, is also recommended. Further, in breeding situations where particular sire and queen combinations have resulted in cases of FIP across multiple litters, retiring of one or both cats from the breeding programme should be considered in case they are conferring increased genetic risk of FIP.

The FIP vaccine (Felocell® FIP, Zoetis), where available, is not recommended for use in cats under 16 weeks age or considered to be of benefit to cats that are already FCoV antibody-positive cats. It is therefore of limited usefulness as initial exposure to FCoV is considered to be much earlier than 16 weeks in most cases. Further, as current serological tests are unable

to differentiate vaccinated from naturally-exposed cats, it is of no benefit if trying to maintain a FCoV-free household. The FIP vaccine is either 'not recommended' or considered 'non-core', under current AAFP, ABCD, and WSAVA vaccination guidelines (and not even discussed in the BSAVA vaccination guidelines).

Box 3*: When one cat gets FIP, what to do with the other cat(s) in the household?*

* Where more than one cat is being considered (i.e. the one with FIP, an its in-contact), an accurate diagnosis becomes more important in order to guide advice

* In-contact cats will be at slightly increased risk cf. the general population, particularly if they are direct siblings (estimated 2x risk), due to shared viral, environmental and possibly genetic factors – but none of these can be changed at time of diagnosis!

* Whilst cats can pass FCoV that causes intestinal infection between each other, they are not thought to be able to horizontally pass the mutated FCoV that directly causes FIP between themselves (i.e. the mutation is a spontaneous event that happens within individuals). Given that the cat with FIP would have had historical intestinal infection with FCoV, it is likely that the other cat(s) in the household would have been infected historically too

* Removal or isolation of a cat with (suspected / proven) FIP from a household is not indicated, and would likely negatively impact on the sick cat

* As the household environment (inc. fomites) is likely contaminated with FCoV (particularly if there are 5+ cats in the household, to maintain continuous infection) – improved hygiene (particularly litter tray-associated) is strongly recommended

* As stress is associated with the development of FIP – reducing household stress (e.g. due to conflict, overcrowding, or continued breeding) is recommended, as is deferral of non-essential, elective procedures (e.g. microchipping; neutering)

* As FCoV can survive under appropriate conditions for up to 7 weeks in the environment and the loss of a house-mate will be stressful for the remaining cats (and therefore may temporarily induce FCoV shedding in carriers) – immediate 'replacement' of the deceased cat is strongly discouraged (for at least 3 months). These replacements may be naïve to the FCoV isolate circulating in the household, are typically young (i.e. in the highest risk category for going on to develop FIP following exposure to the virus), may share some genetic risk factors (i.e. if from the same source as the deceased cat), and may well cause stress and conflict within the household (i.e. owners often have the misconception that the remaining cat(s) need the company of another cat).

* Faecal shedding by remaining cats (e.g. by weekly faecal PCRs on 3-4 occasions) could be considered prior to the introduction of a new cats, but this would not completely eliminate risk (as shedding is intermittent), and a significant number of cats are infected with FCoV from their original household such that the incoming cat may have already been exposed to FCoV)

SUMMARY

FIP is a common differential for disease in, often younger, cats. Obtaining a definitive diagnosis by minimally-invasive means can be difficult, and a balance of probability might need to be used to guide further testing. Although currently treatment is limited, novel anti-viral agents show real promise for the future.

ADDITIONAL READING:

Tasker, S. (2018) Diagnosis of feline infectious peritonitis: Update on evidence supporting available tests. *Journal of Feline Medicine and Surgery* **20** 228-243

The most up to date version of the ABCD guidelines on Feline Infectious Peritonitis (Addie et al. 2019) are available online www.abcdcatsvets.org/feline-infectious-peritonitis/

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682 **FIGURES**

683 **Figure 1** Schematic diagram of the feline coronavirus genome (approximately 29,250
684 nucleotides in length excluding the polyA tail) with component genes and nucleotide scale.
685 Note that over two thirds of the genome comprises the gene that encodes non-structural
686 polyprotein 1.

687

688 **Figure 2** Schematic drawing of a feline coronavirus virion with relative position of structural
689 proteins and genomic single-stranded RNA (ssRNA) indicated. The spike glycoprotein trimers
690 project from the surface of the virus, resulting in the 'crown'-like appearance when viewed
691 under transmission electron microscopy from which they are named.

692

693 **Figure 3** Numerous granulomas (black arrows) within a sectioned kidney from a two-year-
694 old male neutered cat with feline infectious peritonitis. The cat was presented with an acute
695 history of lethargy and inappetence. Physical examination revealed a thin body condition,
696 pale and icteric mucous membranes, subtle unilateral anterior uveitis and bilateral
697 renomegaly. Blood analysis documented severe anaemia (haematocrit 7.8%; reference
698 interval [RI] 27-47%), hyperglobulinaemia (83g/L; RI 21-51g/L) and jaundice (31µmol/L; RI
699 <10µmol/L). On post-mortem examination a moderate volume of ascites was present,
700 alongside small volume pleural and pericardial effusions. Both kidneys were enlarged with
701 margins distorted by vascularised mass lesions. Pyogranulomatous lesions were found on
702 histopathology of samples collected throughout the body (including the iris), with presence
703 of feline coronavirus confirmed with reverse-transcriptase PCR and immunohistopathology.

704

Figure 4 Ultrasonographic images of two cats with feline infectious peritonitis. The first cat (A) had a large mid-abdominal mass on examination, which was identified as being mesenteric lymph node using ultrasound (yellow calliper). The second cat (B) also had enlarged abdominal lymph nodes (yellow calliper); however, a small volume of ascites was also visible (white arrow). Ultrasound enabled guided sampling of both the enlarged lymph nodes and the ascites.

Figure 5 Photo (A) of a 6-month-old male entire Ragdoll, presented with a 48-hour history of altered behaviour and a 24-hour history of hind-limb paresis, reduced appetite, nystagmus and mild head tilt. Neurolocalisation was most consistent with central vestibular syndrome, mostly likely a result of cerebellar disease. Routine haematology and serum biochemistry were unremarkable. MRI (B; T2-weighted midline sagittal view) revealed severe hydrocephalus with dilation of the entire ventricular system of the brain and secondary herniation of the cerebellum, the latter likely accounting for the majority of clinical signs. Ultimately, the presence of feline coronavirus was confirmed with reverse-transcriptase polymerase chain reaction of cerebrospinal fluid, alongside histopathology and immunostaining of meningeal tissue samples confirming a diagnosis of feline infectious peritonitis.

Figure 6. Feline infectious peritonitis is a major differential for uveitis, manifestations of which include: changes in iris colour, thickness and texture; dyscoria (abnormal shape of the pupil); anisocoria (unequal pupil sizes); sudden loss of vision; hyphaema; keratic precipitates (‘mutton fat’ deposits on the ventral corneal endothelium); chorioretinitis; retinal detachment; and aqueous / vitreous flare. These changes may be subtle and unilateral (A;

mild iridial changes, aqueous flare and keratic precipitates [white arrow] of the right eye in a 8-month-old Chinchilla Persian with pyrexia of unknown origin; images courtesy of Caroline Smith), or bilateral and severe (B; bilateral severe fibrinous, flocculent, aqueous flare limiting examination of the interior of the eye; images courtesy of Vim Kumaratunga), where assessment of the retina is possible changes may be present there too (C; severe bilateral chorioretinitis including haemorrhage and granulomata [pink and red arrow]; aqueous flare and retinal oedema results in the image appearing to be out of focus; images courtesy of Vim Kumaratunga)

Figure 7 Diagnostic approach to cats with suspected FIP

Figure 8 Although effusions from cats with feline infectious peritonitis are typically clear, viscous with a tendency to froth when agitated, and with a slightly yellow tinge, reflecting a low cellularity (<5 x10^9/L total nucleated cell count), high concentration of predominantly inflammatory proteins (>35g/L), and patient jaundice respectively, their gross appearance can be variable.